BUDAPEST UNIVERSITY OF TECHNOLOGY AND ECONOMICS
FACULTY OF CHEMICAL ENGINEERING AND BIOENGINEERING
OLÁH GYÖRGY DOCTORAL SCHOOL

Physical and chemical aspects of the application of PLA in practice

Thesis booklet

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2022

Introduction

The interest in the use of biopolymers has increased considerably in recent years. The targeted areas are the packaging industry, agriculture and medical devices; the latter has been extensively studied especially in the field of drug delivery. Probably poly(lactic acid) (PLA) is the polymer used in the largest quantity in such applications, because of its availability, reasonable price and competitive overall properties. PLA is produced from renewable materials, it is biodegradable, but also has large stiffness and strength. However, several drawbacks limit the applications of PLA, including its water sensitivity, slow crystallization, and its glass transition temperature is low and its properties change with time relatively fast. PLA products become quite brittle with time because of the factors listed above including physical ageing that happens spontaneously and leads to the change of properties.

In order to improve the properties to reach increasing demands, nanocellulose is a promising candidate for the reinforcement of PLA leading to fully biodegradable composites. A major problem encountered during the preparation of such nanocomposites is homogeneity. Nanofillers tend to aggregate which deteriorates the properties of the composites prepared. A successful way to improve dispersion and achieve the required homogeneity is the use of the Pickering emulsion technique [1]. Although biopolymers might not solve the problem of environmental pollution completely, they may offer a viable alternative in certain areas. Some of these polymers degrade biologically either in the environment or in a compost thus decreasing the amount of plastic waste causing pollution. Serious debate is going on occasionally questioning the use of biopolymers since the biodegradation of bioplastics is a complicated process.

In view of the considerations mentioned above it is obvious that several practical issues must be studied and resolved during the use of PLA. This work embraces two collaborations dedicated to cope with current issues related to the practical use of PLA. The first is the joint research initiated by the Faculty of Pharmacy at the University of Szeged and our group. PLA-based medical scaffolds were prepared by electrospinning which contained active drugs for the treatment of the periodontitis. The possible use of PLA in practical applications and especially as structural materials called the attention to the importance of structural changes during their use and especially to the effect of physical ageing on properties. Because of the importance of the phenomenon, we studied and modelled the process in detail. Recently, Xiaofeng Sui's group from the Dong-

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[1] Zhang, Y., Wu, J., Wang, B., Sui, X., Zhong, Y., Zhang, L., Mao, Z., Xu, H. *Cellulose* **24**, 3313-3322 (2017)

hua University reported the use of the Pickering emulsion approach for the production PLA/cellulose nanocomposites with better dispersion and reinforcement than before. We contributed to the work by the analysis of correlations between structure and properties first and then turned our attention to the treatment of the waste of PLA and its composites. This thesis mainly focuses on issues related to the integrated life cycle of PLA in practice including its potential medical use, physical ageing during application, correlations between structure and properties, and its enzymatic degradation at the end of its life.

1. Background

The pharmaceutical industry shows increasing interest in the electrospinning technique due to its extraordinary advantages, such as large processing flexibility, controlled drug release kinetics and topical/systemic therapies compared with traditional drug formulations [2]. However, many factors influence the efficiency of such devices. The physical-chemical properties of the polymer, the drugs and the solvents greatly influence interactions and drug release kinetics. The compatibility of the drug and the solvent as well as the drug and the polymer, polymer/solvent interactions both in the spinning solution and in the fibers, as well as the evaporation of the solvent, all impact the location of the drug, encapsulation and the solid-state characteristics of the formulation [3].

Nevertheless, little attention is paid to the physical-chemical aspects of the preparation or the drug release process. The authors usually assume that the composition of the fibers is the same as that of the spinning solution and the fibers are homogeneous. Homogeneous drug distribution is not assumed, because the particles of the drug are often observed on the surface of the fibers. The phase separation of the polar drug and the presence of the particles usually leads to a burst like delivery, which is said to be followed by a slow, controlled release. The papers on electrospun biopolymer devices containing Amox unanimously agree on this two-step process irrespective of differences the experimental details [4]. However the interaction of the components, mutual miscibility, the structure of the matrix, the distribution or partitioning of the active component are mentioned occasionally but rarely investigated, although all must influence the time dependence, extent and efficiency of drug delivery very much.

[2] Khoshnevisan, K., Maleki, H., Samadian, H., Shahsavari, S., Sarrafzadeh, M.H., Larijani, B., Dorkoosh, F.A., Haghpanah, V., Khorramizadeh, M.R. *Carbohydr. Polym.* **198**, 131-141 (2018)

[3] Valo, H., Peltonen, L., Vehviläinen, S., Karjalainen, M., Kostiainen, R., Laaksonen, T., Hirvonen, J. *Small* **5**, 1791-1798 (2009)

[4] Sofokleous, P., Stride, E., Edirisinghe, M. *Pharm. Res.* **30**, 1926-1938 (2013)

In addition, several factors influence the fundamental properties of this polymer during use and the effect of some of them is very disadvantageous. Because of the relative low glass transition temperature of PLA, its physical ageing is fast. Considering the increasing importance of PLA, it is not very surprising that quite a few studies focused on its physical ageing [5,6]. The majority of these were carried out by using thermal analysis, DSC. PLA samples are usually annealed at different temperatures and subsequently they are heated up in the DSC pan to determine the glass transition temperature as well as the enthalpy of the transition. The changes in these characteristics depend on the temperature of annealing, but also on other factors, e.g. cooling rate, which are much less specified in the papers published [7]. Most of the results showed an increase in glass transition temperature (T_g) with annealing time, which is almost invariably explained with decreased free volume and molecular mobility. However, occasionally a decrease is observed in the glass transition temperature of PLA [8,9], which is more difficult to explain and is rarely done. The consequence of physical ageing on the mechanical properties of PLA products and generally on practice is investigated or mentioned very rarely. Several studies were done on PLA fibers showing the increase of tensile yield stress and tensile strength with ageing time, the appearance of a maximum and decrease at longer times [10]. The increase is in accordance with the free volume theory, but a plausible explanation was not offered for the maximum and the subsequent decrease. Although the physical ageing of PLA is an important phenomenon with a large impact on its application, numerous contradictions surround it, which have not been studied and explained satisfactorily yet.

The concern about the effect of physical ageing on the behavior of PLA indicates that considerable improvement in processing technology, initial design and customer service of the products are required in industrial applications. The various requirements of the market and newly established environmental laws allow only the use of biopolymers with high performance. The modification of PLA by reinforcement is one way to improve performance and meet the requirements. The regeneration of cellulose from a solution by the coagulation with a non-solvent is an important pathway for the preparation and industrialization of cellulose materials. Materials fabricated directly from a cellulose solution via a

[5] McKenna, G.B., Simon, S.L. *Handbook of thermal analysis and calorimetry* 3, 49-109 (2002)

[6] Pan, P., Zhu, B., Inoue, Y. *Macromolecules* **40**, 9664-9671 (2007)

[7] Liao, K., Quan, D., Lu, Z. *Eur. Polym. J.* **38**, 157-162 (2002)

[8] Cai, H., Dave, V., Gross, R.A., McCarthy, S.P. *J. Polym. Sci., Part B: Polym. Phys.* **34**, 2701-2708 (1996)

[9] Tsuji, H., Sawada, M. *J. Appl. Polym. Sci.* **116**, 1190-1196 (2010)

[10] Chang, G., Zhang, Y., Deng, Y., Liu, Q., Bao, W. *New Chem. Mater.* **41**, 129-131 (2013)

physical regeneration process possess unsurpassed physical and chemical properties, inherent renewability and sustainability and thus they exhibit astonishing impacts and benefits for society [11].

Two techniques are widely adopted for the preparation of polymer/cellulose nanocomposites. The first is solvent casting by the evaporation of water or an organic solvent [12]. The second is the direct extrusion of the matrix polymer containing the desired amount of freeze-dried cellulose nanoparticles [13]. However, the main difficulty related to the preparation and quality of such composites is the dispersion of cellulose particles in hydrophobic polymers. Much effort has been made to improve the dispersibility of cellulose particles in polymer matrices, which are mainly devoted to surface modification [14]. Modified cellulose was more readily incorporated into matrices, but their reinforcing effect was compromised due to the loss of H bond-forming hydroxyl groups resulting in the deterioration of the mechanical properties of the final composites, including PLA/cellulose composites.

Raw materials must be transformed into useful products in industry at larger amounts and in the case of thermoplastics, this is usually done by melt processing. Accordingly, the knowledge of the rheological properties of the materials and the effect of modification on them are very important for practice. Nanofillers usually increase melt viscosity and the association of the particles may lead to the formation of a network structure, even aggregation above a critical amount of the filler. Although the rheological characteristics of PLA/nanocellulose composites have been reported many times [15], a thorough analysis is often lacking. Such analysis has been carried out for polypropylene composites [16], and even though one can find a few articles in the literature which aim at the quantitative characterization of the rheological behavior of PLA based composites [17], the models presented in them may yield results that are difficult to interpret. The limited reliability of these results, however, often hinders the accurate prediction and optimization of the extrusion process [18].

[11] Wang, S., Lu, A., Zhang, L. *Prog. Polym. Sci.* **53**, 169-206 (2016)

[12] Lin, N., Huang, J., Chang, P.R., Feng, J., Yu, J. *Carbohydr. Polym.* **83**, 1834-1842 (2011)

[13] Goffin, A.-L., Raquez, J.-M., Duquesne, E., Siqueira, G., Habibi, Y., Dufresne, A., Dubois, P. *Biomacromolecules* **12**, 2456-2465 (2011)

[14] Pei, A., Zhou, Q., Berglund, L.A. *Compos. Sci. Technol.* **70**, 815-821 (2010)

[15] Dhar, P., Gaur, S.S., Soundararajan, N., Gupta, A., Bhasney, S.M., Milli, M., Kumar, A., Katiyar, V. *Ind. Eng. Chem. Res.* **56**, 4718-4735 (2017)

[16] Khoshkava, V., Kamal, M.R. *ACS Appl. Mater. Interfaces* **6**, 8146-8157 (2014)

[17] Zare, Y., Rhee, K.Y. *Composites, Part B* **156**, 100-107 (2019)

[18] Kamal, M.R., Khoshkava, V. *Carbohydr. Polym.* **123**, 105-114 (2015)

Despite thorough investigations and comprehensive research carried on PLA and its composites, environmental pollution still cannot be ignored completely because of the serious limitations related to their recycling and degradation at the end of their life. Because of its importance, the enzymatic degradation of PLA, the aliphatic polyester biopolymer produced and used in the largest quantities presently, has been widely investigated by various groups. The results of the experiments showed that the most efficient catalysts for the enzymatic degradation of PLA are the alkaline proteases [19] and the commercial product Proteinase K is extensively used for the purpose. The studies included many aspects of the enzymatic degradation of PLA including the effect of enzyme concentration [20], temperature [21], ion concentration of the buffer used as degradation medium [22], the molecular weight [23], stereochemical composition and crystallinity of PLA [24]; even the effect of physical ageing on the rate of degradation [25] as well as the influence of various additives were studied to smaller or larger extent [26]. Unfortunately, the results of the experiments were evaluated only qualitatively, degradation kinetics has not been analyzed quantitatively by the use of appropriate models. Moreover, less attention was paid to the changing acidity of the degradation medium and to the occasional denaturation of the enzyme.

The thesis aims at the practical issues in the use of PLA from physical and chemical aspects. The first study relates to the medical application, we approached controlled drug release from a new perspective by focusing on the physical-chemical aspects of release from devices prepared by the PLA electrospun fibers. For further use in industry, especially in the engineering applications, the durability of products is usually required, the change of properties with time must be considered. Then we studied the factors and consequences of PLA physical ageing. Besides, the single-use product also has a large quantities capacity of the production of PLA, mainly in packaging industry. Due to the various demands of the market and newly established environmental laws, biocomposites becomes a trendy topic in research. So we prepared the cellulose reinforced PLA composites via the Pickering emulsion

[19] Martin, R.T., Camargo, L.P., Miller, S.A. *Green Chem.* **16**, 1768-1773 (2014)

[20] Yamashita, K., Kikkawa, Y., Kurokawa, K., Doi, Y. *Biomacromolecules* **6**, 850-857 (2005)

[21] Bajorath, J., Saenger, W., Pal, G.P. *Biochim. Biophys. Acta, Protein Struct. Mol. Enzymol.* **954**, 176-182 (1988)

[22] Hegyesi, N., Zhang, Y., Kohári, A., Polyák, P., Sui, X., Pukánszky, B. *Ind. Crops Prod.* **141**, 111799 (2019)

[23] Tsuji, H., Miyauchi, S. *Biomacromolecules* **2**, 597-604 (2001)

[24] Reeve, M.S., McCarthy, S.P., Downey, M.J., Gross, R.A. *Macromolecules* **27**, 825-831 (1994)

[25] Cai, H., Dave, V., Gross, R.A., McCarthy, S.P. *J. Polym. Sci. B: Polym. Phys* **34**, 2701-2708 (1996)

[26] Luzi, F., Fortunati, E., Puglia, D., Petrucci, R., Kenny, J., Torre, L. *Polym. Degrad. Stab.* **121**, 105-115 (2015)

approach, and studied the structure-property correlation and rheology. After discussing pratical issues and possibilities in different uses, we paid attention to the post treatment of PLA products, and analyzed the determining factors on enzymatic degradation of PLA quantitatively.

2. Experimental

The five PLA grades were supplied by NatureWorks (USA). Their weight and number average molecular weight (Mw and Mn) as well as D-lactide content are collected in **Table 1**.

Table 1 *Molecular weight and stereoregularity of the Ingeo PLA grades used in this study*

Ingeo grade	M_n (g/mol)	M_w (g/mol)	M_w/M_n	D-lactide (%)
3260HP	44600	86000	1.93	0.5
3251D	50700	89000	1.76	1.2
3100HP	63500	125900	1.98	0.5
2003D	64800	129000	1.99	4.3
4032D	85700	167100	1.95	2.0

PLA fibers containing various amounts of amoxicillin (Amox) were prepared by electrospinning, and the solvent was removed in the oven afterward. Electrospun fiber mats taken directly from the foils and round, compressed disks were used for characterization, as well as the analysis of release kinetics and microbial activity. The polymer was dried in a vacuum oven before processing. Standard specimens (ISO 527 1A) were produced by injection molding. Physical ageing was done by storing the specimens in a room with controlled temperature and humidity for different conditioning times prior further measurements. The cellulose reinforced PLA composites were prepared by the Pickering emulsion approach published in the literature [27]. Around 40 g composite powder was compression molded into 1 mm thick plate. The composites contained 0.5, 1.0, 1.5, 2.0, 3.0 and 5.0 wt% regenerated nanocellulose (RC). The rheological measurements of the composites were carried out in the oscillatory mode using a rotational viscometer.

The enzymatic degradation studies were carried out by using five types of PLA compressed plates, the samples were placed into vials and the enzyme solutions. The vials were shaken at 37 °C in an incubator afterwards. Individual

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[27] Zhang, Y., Cui, L., Xu, H., Feng, X., Wang, B., Pukánszky, B., Mao, Z., Sui, X. *Int. J. Biol. Macromol.* **137**, 197-204 (2019)

samples were investigated in each case. In the experiments, the samples were taken from the vial after a certain degradation time, washed with water, wiped and weighed. Enzyme concentration was 0.01, 0.02, 0.04, 0.08, 0.1, 0.2, 0.3, 0.4 and 0.5 mg/ml in the first series of measurements. The volume of the degradation medium was 5 ml in this and in all other series except in which the volume of the degradation medium was changed. In another series degradation was done at various temperatures, at 23, 37, 45, 50 and 65 °C. The measurements were done twice at 37 °C in order to check their reliability. Enzyme concentration was 0.2 mg/l. The same enzyme concentration was used in the series in which the influence of degradation volume was studied; volumes of 5, 10, 20, 30 and 40 ml were used in the experiments. The degradation medium was changed daily in a series comparing the effect of changed/unchanged medium and also in the series studying the effect of molecular weight on the rate of degradation.

Gel permeation chromatography was used for the determination of the molecular weight of the polymers studied (**Table 1**). The structure of the materials prepared was characterized by using scanning electron microscope (SEM). Samples were fractured at liquid nitrogen temperature and then a thin gold layer was sputtered onto the fractured surface prior to the SEM study. The morphology of RC was characterized using transmission electron microscopy. The transitions, crystalline structure and crystallization behavior of the samples was studied by differential scanning calorimetry (DSC) and X-ray diffraction (XRD) measurements. The mechanical properties of specimens were characterized by tensile testing using an Instron 5566 apparatus at 115 mm gauge length. The solubility of amoxicillin in solvents or solvent mixtures, the release of the drug, and the light transmission of the compression molded plates of the polymer in were determined by UV-Vis spectroscopy (UV-Vis).

3. Results

The release of Amox from the PLA disks prepared in this project is presented in **Figure 1** as a function of time. Release increases rapidly initially and then approaches a plateau, indicating maximum release, which is around 50-60 % in this case. The plateau is reached in approximately two-three days and we did not observe further release, at least in the time scale of the experiment. The partial release of the drug is in accordance with the considerations presented before, i.e. with the encapsulation of the drug into the PLA fibers resulting in slow or negligible release, but also by its physical loss during the handling of the disks. The extent of release depends also on the dissolution medium, it is somewhat larger into the saline solution than water. Such an effect of the dissolution medium has been observed before, occasionally very small amount of Amox was released into all media. The fact that a constant value of release is

reached needs further consideration. Practically all reports related to the release of Amox from electrospun fibers or related devices claim that release takes place in two steps, a burst like rapid release and then prolonged release going on for a long time. Unfortunately, in the case of PLA/Amox fibers, although a two-stage process is claimed, the experimental evidence does not support it in our case. Amox release reaches a constant value in a couple of days instead of a prolonged release. The basis of the two-step hypothesis and prolonged drug release is the assumption of slow diffusion of the encapsulated drug from inside the fi-

Figure 1 *Burst like drug release from PLA disks prepared from electrospun fibers containing 1.23 % Amox.*

bers. However, we must thoroughly consider here the possibility and rate of diffusion. The volume of the mapped cuboid of Amox three-dimensional structure is 0.544 nm^3. The molecule must move in the amorphous phase, through the free volume of the polymer. The size of the individual voids creating the free volume was determined as 0.124 nm^3. The dissimilarity in the two values indicates very slow diffusion at most thus questioning the hypothesis of a two-step release. However, one may consider the possible occurrence and effect of hydrolysis. The hydrolytic degradation of PLA is slow at neutral pH, but larger or smaller pH as well as other factors, like the catalytic effect of some components, may lead to the degradation of the polymer. As an effect of hydrolysis the medium usually becomes more acidic, which accelerates degradation further. The degradation of the polymer may result in the slow release of drugs leading to the second slow step observed by many researchers. Unfortunately, composition and conditions are rarely specified and the possibility of degradation usually is not checked, thus the reason for the slow release is difficult to identify. Similarly, the method of fabrication may also influence the rate of release, but a detailed study has not been made on this effect either, at least we are not aware of any such study. Nevertheless, we can clearly state that physical-chemical issues play an important role in the determination of the extent and mode of drug release, indeed.

Injection molded specimens were prepared from PLA and their properties were determined as a function of time to study physical ageing. The mechanical testing of specimens showed that properties change rapidly with time. The stiffness of the specimens increases considerably, while their deformability decreases drastically from 250 % after injection molding to a few percent after
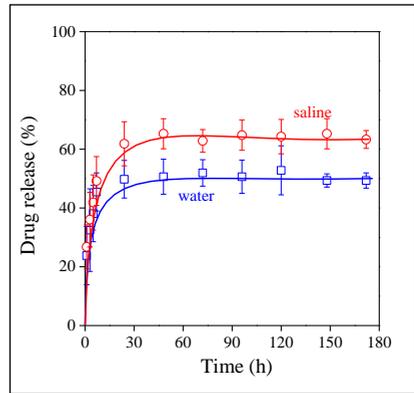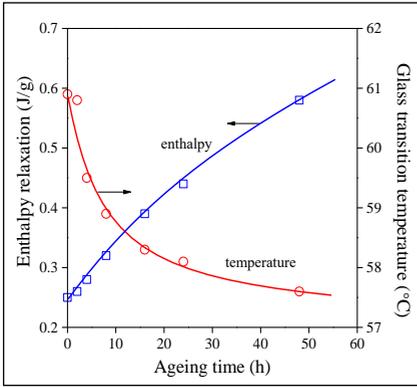
Figure 2 *The influence of ageing time on the intensity of enthalpy relaxation and the glass transition temperature of PLA. Symbols: (○) T_g, (□) enthalpy relaxation.*

less than a day of ageing. Thermal analysis showed that both relaxation enthalpy and the change in the T_g of the polymer increased with time (**Figure 2**). T_g decreased with increasing ageing time that could not be explained by the generally accepted approach of decreasing free volume. The analysis of literature data showed that the decrease or increase of T_g depends on the temperature of ageing and on the rate of cooling. T_g decreases at relatively low ageing temperatures, while it increases when ageing temperatures are closer to the T_g of the polymer. Besides the decrease of free volume, the development of internal stresses also plays a role in the determination of the glass transition temperature. Internal stresses result in a decrease of T_g, while decreased free volume leads to an increase. Internal stresses determine deformation and failure mechanism as well; large stresses lead to crazing/cracking and finally to brittle failure, which may hinder the application of PLA in many areas.

PLA/RC composites with different compositions were prepared successfully by the Pickering emulsion approach. The mechanical properties of neat PLA and the PLA/RC composites are shown in **Figures 3** and **4** as a function of RC content. The tensile strength of the composites has a maximum at the RC amount of 0.5 wt%, thereafter it decreases with increasing RC content. Tensile strength changes in the range of 51 and 43 MPa upon the addition of a relatively small amount of RC up to 5 wt%. Possibly the network formation or aggregation of RC (detected by SEM images) above 1.5 wt% RC content result in the smaller tensile strength compared to the neat PLA. On the other hand, the composites containing 0.5 and 1 wt% RC show larger tensile strength due to the good dispersion of RC and the results indicate good adhesion between RC and the PLA matrix. The extent of reinforcement can be estimated quantitatively with the help of a simple model. The dependence of tensile strength on filler content can be expressed by **Equation 1**:

$$\ln\sigma_{Tred} = \ln\frac{\sigma_T(1 + 2.5\varphi)}{\lambda^n(1 - \varphi)} = \ln\sigma_{T0} + B\varphi \tag{1}$$

where σ_T and σ_{T0} are the true tensile strength of the composite and the matrix,
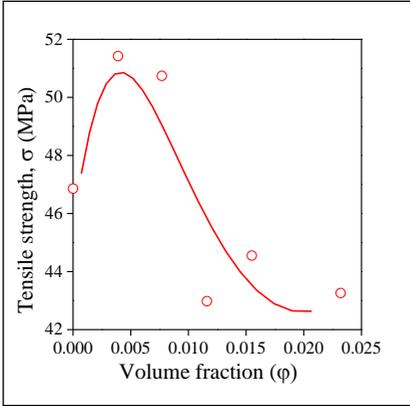
Figure 3 *Plots of tensile strength of PLA/RC composites against their RC content.*
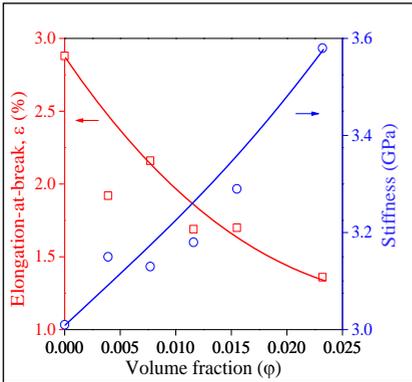

**Figure 4** *Elongation-at break (□) and stiffness (○) of PLA/RC composites plotted against RC content.*

respectively, λ is relative elongation, n is a parameter expressing the strain hardening tendency of the matrix, φ is the volume fraction of the fiber and B is related to its relative load-bearing capacity, i.e., to the extent of reinforcement, which depends among other factors also on interfacial interaction. The natural logarithm of the reduced tensile strength of the composites is plotted against RC content in the form indicated by **Equation 1**. The plot apparently shows relatively constant values. We may speculate that the small load-bearing capacity of RC is the result of poor strength of interaction between the components. However, structural effects, i.e. aggregation and network formation must be taken into account here. Accordingly, **Equation 1** was fitted to the values of the composites containing 0.5 and 1.0 wt% RC, because the model is not valid in the presence of the structural effects mentioned. The slope of the line is 13.9 indicating good dispersion and large reinforcing effect because of the strong interaction of the components.

With increasing volume fraction of RC, the elongation-at-break of the composites decreases owing to the strong interactions. Compared to neat PLA, the stiffness of the composites increased with the addition of RC. The effect of interactions, and even that of structure, is much smaller here, because Young's modulus is determined at very small deformations and thus does not depend very much on the factors mentioned.

The main reason of modeling is to gain better insight into the rheological behavior of materials through the interpretation of the physical meaning of model parameters. Viscosity was modelled with the modified Carreau-Yasuda
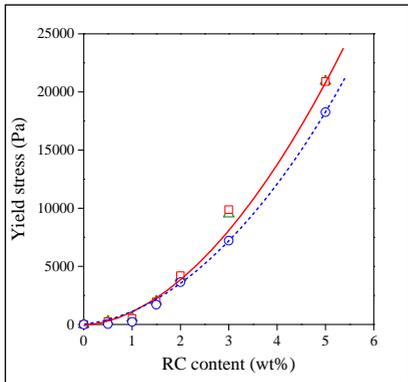
Figure 5 *Dependence of predicted yield stress on RC content. Symbols: (△) Khoshkhava [16], (□) Berzin [28], (○) this work.*
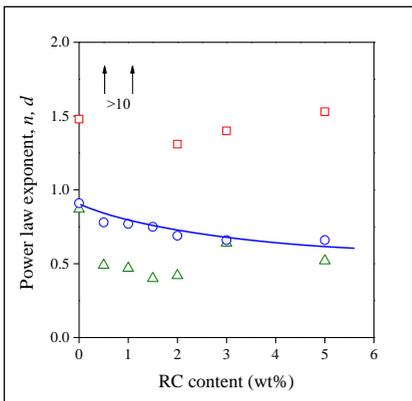
Figure 6 *Effect of RC content on the power law parameters, n and d, determined by the rheological models studied in this work. Symbols: (△) Khoshkhava [16], (□) Berzin [28], (○) this work.*

[16] and the modified Cross models [28] and a new model was proposed as well. A common feature of the three models used in this study is yield stress indicating aggregation or the formation of a cellulose network. Yield stresses derived from the models are presented in **Figure 5** as a function of RC content. The tendency is the same for all three models and the values are very close to each other; the model developed in this work yields somewhat smaller values, but the difference is insignificant. We may conclude that the yield parameter of the equations used offers proof for network formation and expresses its extent in quantitative terms.

Much more contradictory is the composition dependence of the other parameters determined and thus also their interpretation. The power law exponent is plotted against RC content in **Figure 6**. The value of the exponent should be between 0.1 and 1.0 and we expect a decrease with increasing amount of the reinforcement, i.e. increased sensitivity to shear, stronger shear thinning. Quite surprisingly, the power law exponent derived from the modified Carreau equation [16] is very large, values larger than 10 were obtained at certain compositions, which is difficult to understand. Somewhat simpler models with fewer adjustable parameters seem to be more reasonable in this case. All three predict yield stress well, but only the new model gives reasonable values for the power law exponent.

In the enzymatic degradation study of PLA plates. The model describing

[28] Berzin, F.; Amornsakchai, T.; Lemaitre, A.; Castellani, R.; Vergnes, B. Polym. Compos. 2019, 40, 4519-29.

the kinetics of enzymatic degradation quantitatively must consider all three steps. However, since the first step, the adsorption of the enzyme on the substrate is completed in a short time, we ignore it here in order to simplify the treatment. Such a model has been developed in our lab recently and was successfully used to describe the enzymatic degradation of polycaprolactone and PLA [22,29]. If we follow degradation by the loss of sample weight, the rate of degradation in the linear stage, as predicted by the Michaelis-Menten model [30], can be described by first order kinetics. However, we must take into account the denaturation of the enzyme as well, which results in the decrease of degradation rate. We assume that denaturation proceeds also according to first order kinetics and after the necessary mathematical operations, we obtain the correlation describing the time dependence of degradation, i.e. weight loss in the following form

$$m(t) = v_d \, \tau \, [1 - \exp{(-t/\tau)}] \qquad (2)$$

where v_d gives the initial rate of degradation at zero time, τ is a time constant which is related to the rate of denaturation, and the pre-exponential term $A = v_d \tau$ equals the loss of mass at infinite time, i.e. the amount of polymer degraded by the enzyme. If denaturation is absent, like in the case of changing degradation medium, mass loss changes linearly with time, it is constant in the linear regime of degradation as proposed by the original Michaelis-Menten model [29].

The quantitative analysis of results by **Equation 2** helped the determination of factors influencing the enzymatic degradation of PLA proved that Proteinase K degrades the polymer very efficiently. The rate of degradation increases considerably up to 0.1 mg/ml enzyme concentration, but remains constant at larger values. Temperature has an optimum at around 50 °C that is somewhat higher than the 37 °C extensively used in the literature as the most advantageous value. If degradation occurs in the same medium throughout the process, the formation of lactic acid results in the sharp decrease of pH and finally in the denaturation of the enzyme. The dropping of pH below 5 slows down and finally stops degradation completely. The daily change of the medium results in degradation with a constant rate and the entire amount of polymer can be decomposed mainly into monomer or smaller oligomer fragments. The most convenient way for the enzymatic degradation of PLA would be the continuous adjustment of the acidity of the medium, which can be done by various means. The molecular weight of the polymer influences the rate of degradation only slightly, degradation rate decreases with increasing molecular weight. The effect of stereoregularity on the rate of degradation could not be determined unambiguously because the range studied was not sufficiently wide. Nevertheless, the use of appropriate

[29] Polyák, P., Dohovits, E., Nagy, G.N., Vértessy, B.G., Vörös, G., Pukánszky, B. *Int. J. Biol. Macromol.* **112**, 156-162 (2018)
[30] Johnson, K.A., Goody, R.S. *Biochemistry* **50**, 8264-8269 (2011)

kinetic models allowed the quantitative analysis of the results and the unambiguous determination of the effect of several factors on the rate of the enzymatic degradation of PLA.

4.   Thesis points

1.   As a result of the detailed study of the physical-chemical aspects of drug release from drug containing electrospun PLA devices we showed that because of the limited solubility of the drug in the components and that of the components in each other, the composition of the various phases changes during production and only a part of the drug is located within the fibers, the rest can be found among the fibers.

2.   We pointed out the first time that contrary to claims of a two-step release of amoxicillin form electrospun PLA fibers, the second, slow process is less probably because the size of the drug is larger than the free volume of the polymer and thus the drug is released in a single, burst like process.

3.   We called the attention for the first time to the fact that the widely used free volume theory does not explain sufficiently the physical ageing of PLA and the change of its glass transition temperature with time. We proved that besides the change of free volume the development of internal stresses plays a role as well and they determine also the failure mechanism of the polymer.

4.   By the detailed study of the structure-property correlations of PLA/regenerated nanocellulose composites we established the fact that structural effects play an important role in the determination of composite properties. Regenerated cellulose particles form a network already at a small RC content. The results proved that the main issue of the preparation of PLA/nanocellulose composites is aggregation and we called attention to the fact that even the Pickering emulsion process, otherwise offering much better homogeneity than other techniques, have limitations.

5.   During the study of the rheological properties of PLA/RC nanocomposites, we proposed a new model to describe the shear dependence of viscosity. The comparison of the model to existing ones indicated that all three models used describe the yield stress similarly well, but only the new model offers reasonable values for the power law exponent.

6.   During the detailed study of factors influencing the kinetics of enzymatic degradation of PLA and by using a model developed earlier

in our lab we pointed out that the lactic acid forming during the degradation process denatures the enzyme and stops degradation if the reaction is carried in the same vessel without changing the degradation medium. The daily change of the latter avoids extensive denaturation and allows the complete degradation of the polymer. We called attention to the fact that the continuous adjustment of acidity would be a convenient and efficient way of enzymatic degradation.

5. Possible applications

The joint research with Szeged University and with dentists offers the possibility to develop reliable and effective electrospun scaffolds with controlled drug release for dental therapy. Combined with further toxicity and microbiological tests, valuable information can be acquired to make them promising for future use in clinical treatment. Additionally, our group has applied for international grants together with Professor Sui's group at Donghua University having extensive experience in the modification of polymers. The purpose of the cooperation is to develop biocomposites satisfying the requirements from both environmental aspects and performance. The PLA composites reinforced with cellulose nanofibers prepared successfully via the Pickering emulsion approach might find application in the packaging industry in the near future.

6. Publications

Papers used for the preparation of the Thesis

1. Cui, L., Molnár, J.R., Budai-Szűcs, M., Szécsényi, M., Burián, K., Vályi, P., Berkó, S., Pukánszky, B.: Physical-chemical aspects of the preparation and drug release of electrospun scaffolds, *Pharmaceutics* **13**, 1645 (2021). DOI: 10.3390/pharmaceutics13101645. IF: 6,321; FI: 1. [SGD: 90%]

2. Cui, L., Imre, B., Tátraaljai, D., Pukánszky, B.: Physical ageing of poly(lactic acid): factors and consequences for practice, *Polymer* **186**, 122-140 (2020). DOI: 10.1016/j.polymer.2019.122014. IF: 4,43; FI: 14. [SGD: 100%]

3. Zhang, Y.C., Cui, L., Xu, H., Feng, X.L., Wang, B.J., Pukánszky, B., Mao, Z.P., Sui, X.F.: Poly(lactic acid)/cellulose nanocrystal composites via the Pickering emulsion approach: Rheological, thermal and mechanical properties, *Int. J. Biol. Macromol.* **137**, 197-204 (2019). DOI: 10.1016/j.ijbiomac.2019.06.204. IF: 6,953; FI: 37. [SGD: 100%]

4.   Cui, L., Yi, L., Wang, Y.T., Zhang., Y.C., Polyák, P., Sui, X.F., Pukánszky, B.: Rheology of PLA/Regenerated Cellulose Nanocomposites Prepared by the Pickering Emulsion Process: Network Formation and Modeling, *Mater. Des.* **206**, 109774 (2021). DOI: 10.1016/j.matdes.2021.109774. IF: 7,991; FI: 4. [SGD: 80%]

Manuscripts used for the preparation of the Thesis

1.   Cui, L., Wang, X.J., Hegyesi, N., Wang, Y.T., Sui, X.F., Pukánszky, B.: Quantitative analysis of factors determining the enzymatic degradation of PLA, submitted to the *Int. J. Biol. Macromol. (2022)*

Other publications related to the Thesis

1.   Budai-Szűcs, M., Léber, A., Cui, L., Józó, M., Vályi, P., Burián, K., Kirschweng, B., Csányi, E., Pukánszky, B.: Electrospun PLA fibers containing metronidazole for the treatment of periodontitis, *Drug Des. Dev. Therapy* **14**, 233-242 (2020). DOI: 10.2147/DDDT.S231748. IF: 4,162; FI: 11. [SGD: 10%]

Other publications not related to the Thesis

1.   Józó, M., Cui, L., Botz, K., Pukánszky, B.: Processing induced segregation in PLA/TPS blends: factors and consequences, *Express Polym. Lett.* **14**, 768-779 (2020). DOI: 10.3144/expresspolymlett.2020.63. IF: 4,161; FI: 3. [SGD: 20%]

2.   Imre, B., Kiss, E.Z., Domján, A., Cui, L., Pukánszky, B.: Ring-opening polymerization of ε-caprolactone from cellulose acetate by reactive processing, *Cellulose* **28**, 9103-9116 (2021). DOI: 10.1007/s10570-021-04038-8. IF: 5,044. [SGD: 50%]

3.   Cui, L., Qiang, X.: Clean production for chrome free leather by using a novel triazine compound. *J. Renew. Mater.* **7**, 57-71 (2019). DOI: 10.32604/jrm.2019.00118. IF: 1.67; FI: 3. [SGD: 100%]

4.   Yu, L., Qiang, X., Cui, L., Chen, B., Wang, X., Wu, X.: Preparation of a syntan containing active chlorine groups for chrome-free tanned leather. *J. Clean. Prod.* **270**, 122351 (2020). DOI: 10.1016/j.jclepro.2020.122351. IF: 9.297; FI: 10. [SGD: 100%]